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A distinct pattern of disease-associated single nucleotide polymorphisms in IBD risk genes in a family with Crohn's disease

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Abstract: Recent studies have identified more than 160 inflammatory bowel disease susceptibility loci and provided evidence for genetic heritability in disease pathogenesis. Here we describe a case of a 47-year-old White woman suffering from Crohn's disease (CD), who had four children, two with CD and two with a factor V Leiden variation. We analysed the presence of single nucleotide polymorphisms in several CD susceptibility genes. SNP analysis was carried out using commercially available assays. The female CD patient had a positive inflammatory bowel disease family history. All of the patients had a mild disease course, without fistulae or symptomatic stenosis. The patient was heterozygous for risk variants of the genes encoding nucleotide oligomerization domain 2 (NOD2) and Toll-like receptor 5 (TLR5) and a homozygous carrier of both of the identified protein tyrosine phosphatase nonreceptor type 2 (PTPN2) risk alleles. The CD-affected daughter carried heterozygous risk alleles of the genes encoding TLR5, NOD2 and PTPN2. The son, with the earliest onset of disease in the family at the age of 12 years, was heterozygous for risk alleles of autophagy 16 like 1 (ATG16L1), TLR5, NOD2 and PTPN2. This study reports an interesting pattern of CD-associated single nucleotide polymorphisms in a family with CD. This report clearly supports the observation that genetic variations, especially in genes associated with the innate immune system, contribute to disease onset.

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A distinct pattern of disease-associated single nucleotide polymorphisms in IBD risk genes in a family with Crohn's disease

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Recent studies have identified more than 160 inflammatory bowel disease susceptibility loci and provided evidence for genetic heritability in disease pathogenesis. Here we describe a case of a 47-year-old White woman suffering from Crohn's disease (CD), who had four children, two with CD and two with a factor V Leiden variation. We analysed the presence of single nucleotide polymorphisms in several CD susceptibility genes. SNP analysis was carried out using commercially available assays. The female CD patient had a positive inflammatory bowel disease family history. All of the patients had a mild disease course, without fistulae or symptomatic stenosis. The patient was heterozygous for risk variants of the genes encoding nucleotide oligomerization domain 2 (*NOD2*) and Toll-like receptor 5 (*TLR5*) and a homozygous carrier of both of the identified protein tyrosine phosphatase nonreceptor type 2 (*PTPN2*) risk alleles. The CD-affected daughter carried heterozygous risk alleles of the genes encoding *TLR5*, *NOD2* and *PTPN2*. The son, with the earliest onset of disease in the family at the age of 12 years, was heterozygous for risk alleles of autophagy 16 like 1 (*ATG16L1*), *TLR5*, *NOD2* and *PTPN2*. This study

reports an interesting pattern of CD-associated single nucleotide polymorphisms in a family with CD. This report clearly supports the observation that genetic variations, especially in genes associated with the innate immune system, contribute to disease onset. *Eur J Gastroenterol Hepatol* 26:803–806 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Keywords: autophagy 16 like 1, Crohn's disease, factor V Leiden mutation, nucleotide oligomerization domain 2, protein tyrosine phosphatase nonreceptor type 2, single nucleotide polymorphisms, Toll-like receptor 5

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Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract, which includes Crohn's disease (CD) and ulcerative colitis (UC). CD is characterized by a chronic and transmural inflammation that can occur anywhere in the gastrointestinal tract.

Environmental and genetic factors, as well as aberrant immune responses, have been considered as the major aetiologies of IBD and CD in particular. Evidence suggests that an epithelial barrier defect, coupled with a dysfunctional immune response of the innate as well as the acquired immune system to commensal microbiota, resulting in either excessive upregulation or impaired downregulation of inflammatory events, drives the development of chronic intestinal inflammation [1]. The predispositions are genetically determined, and genetic variations, meaning single nucleotide polymorphisms (SNPs), in more than 160 gene loci have been associated with IBD [2,3].

On the basis of population-based studies and incidence cohorts, it has been well confirmed and demonstrated that there is a heritability for IBD, and relatives of an IBD patient have a significantly higher risk of also developing

IBD as compared with the general population [4]. In CD, a large meta-analysis of genome-wide association studies has identified at least 71 replicated loci (meaning that they have been found in at least two independent studies), such as nucleotide oligomerization domain 2 (*NOD2*), autophagy 16 like 1 (*ATG16L1*), immunity-related GTPase family M protein (*IRGM*), Toll-like receptor 5 (*TLR5*), protein tyrosine phosphatase nonreceptor type 2 (*PTPN2*) and *PTPN22* [5]. However, despite increasing knowledge about the functional relevance of these genes in the cellular and molecular context, the relevance of the presence of these mutations with respect to the onset of IBD is still unclear.

Here we report the case of a 47-year-old White woman suffering from CD, who had four children, two also with CD and two with factor V Leiden, and we analyse the presence of several important CD susceptibility genes by SNP analysis.

Case report

In April 2010, a 44-year-old female patient was admitted for active CD with abdominal pain and diarrhoea. Her current medication was steroids and azathioprine.

The patient was first diagnosed with CD in 2009 at the age of 43 years on the basis of radiological examination, colonoscopy and clinical presentation. She was a persistent smoker with a 30 pack-year history. Upon treatment with steroids and mesalazine (5-ASA) an improvement in disease activity was achieved. The therapy with mesalazine had to be stopped because of a general exanthema. Six months later another acute flare of CD had to be treated with steroids, and maintenance treatment with azathioprine was started. On treatment with azathioprine, stable remission was achieved and is still persistent.

Interestingly, the patient had a positive family history of CD and UC. The father of the patient had been diagnosed with UC and developed colorectal cancer in 1990. The patient is married and has four children. Two of them were also diagnosed with CD and two have a factor V Leiden mutation. The patient's husband and father of her children neither suffers from IBD nor has a factor V Leiden mutation; however, he had undergone a kidney transplantation for end-stage kidney dysfunction due to glomerulonephritis.

One daughter was diagnosed with CD in 2011 at the age of 21 years. One son was diagnosed with CD in 2005 at the age of 12 years. Although both of them developed the disease at a young age, both of them have had a mild disease course so far with only azathioprine as maintenance therapy. Both children with factor V Leiden have had no complications until now. None of the family members have suffered from fistulae or relevant

intestinal stenosis so far (Fig. 1). To the best of our knowledge, the two daughters without CD have not undergone endoscopy.

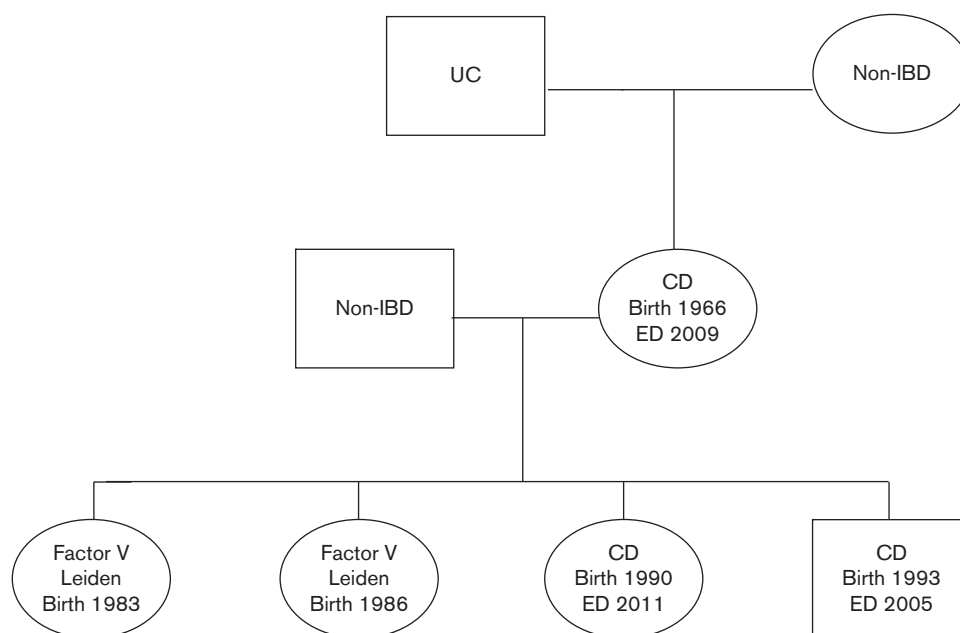
Genotyping

Given the family history, a genetic component of the disease seems obvious. To address the involvement of genetic variations, the patient, her husband and all her children were genotyped for eight common IBD-associated risk alleles in the gene loci encoding *NOD2* (SNP-8, rs2066844; SNP-12, rs2066845), *TLR5* (SNP ID: rs5744168), *ATG16L1* (SNP ID: rs2241880), *IRGM* (SNP ID: rs4958847), *PTPN22* (SNP ID: rs2476601; SNP ID: rs33996649) and *PTPN2* (SNP ID: rs2542151; SNP ID rs1893217). Genomic DNA was isolated from freshly collected blood samples using the QIAmp DNA Blood Minikit (Qiagen, Hilden, Germany). The variants were determined using commercially available SNP Genotyping Assays (Applied Biosystems, Carlsbad, California, USA) and TaqMan Genotyping Master Mix (Applied Biosystems) on a 7900HT Fast Real-Time PCR System using SDS 2.2 Software (Applied Biosystems).

Discussion

The detected variants are all linked to host response to bacteria. *NOD2* is an intracellular receptor recognizing the bacterial cell wall component muramyl dipeptide and is crucially involved in the initiation of an adequate host defence against invading pathogens [6]. Variants of *NOD2* have been identified as a prominent susceptibility factor

Fig. 1



The family tree shows the IBD history of the family in a schematic manner. Male family members are represented by a rectangle, female family members by an ellipse. CD, Crohn's disease; ED, diagnosis; IBD, inflammatory bowel disease; UC, ulcerative colitis.

Table 1 Genetic variations within the family members

	ATG16L1	TLR5	IRGM	PTPN2_1	PTPN2_2	PTPN22_20	PTPN22_30	NOD2_8	NOD2_12
Father	G/G	G/G	G/G	T/T	A/A	G/G	C/C	C/C	G/G
Mother	G/A	G/G	G/G	G/T	A/A	G/G	C/C	C/C	G/G
Daughter	G/G	G/A	G/G	G/G	G/G	G/G	C/C	C/T	G/G
Daughter	G/G	G/A	G/G	G/T	A/G	G/G	C/C	C/T	G/G
Daughter	G/A	G/G	G/G	G/T	A/G	G/G	C/C	C/C	G/G
Son	G/G	G/A	G/G	G/T	A/G	G/G	C/C	C/C	G/G
Son	G/A	G/A	G/G	G/T	A/G	G/G	C/C	C/T	G/G

Line 2 represents the wild-type variant of each SNP. CD-affected individuals are represented in light grey.
CD, Crohn's disease; SNP, single nucleotide polymorphism.

for IBD [7], and loss of function results in defective immune response and ineffective bacterial handling. Interestingly, we only found a variation in the *NOD2* SNP-8, but not in SNP-12.

ATG16L1 is a protein involved in autophagy modulation and the CD-associated T300A allele results in defective autophagy [8,9]. Autophagy is an important cellular survival mechanism, but it is also involved in degradation and clearance of intracellular bacteria and effective antigen presentation. Further, it is linked at a functional level to *NOD2* and both of them can directly interact to control the initiation of the adaptive immune response against invading bacteria [10,11]. Although the presence of the heterozygous *ATG16L1* variation is rather common, even among healthy individuals, we found it only in the son affected with CD, in the healthy father and in one of the daughters without CD.

TLR5 is an extracellular receptor sensing the conserved bacterial product flagellin. Defective TLR5 signalling results in poor proinflammatory response to enteric bacteria and variants are negatively associated with IBD [12]. The heterozygous variation was found in all family members affected by CD and also in one of the daughters without CD.

PTPN2 is a protein that controls proinflammatory signalling cascades, including pathways downstream of *NOD2*, and is involved in autophagy regulation [13]. The two IBD-associated variants result in the expression of a dysfunctional protein that is ineffective in the regulation of proinflammatory signalling pathways [14]. Interestingly, the mother was a homozygous carrier of both of the IBD risk variants. All of her children were heterozygous for both of these variations.

None of the family members had a homozygous or heterozygous variation within the IBD-associated autophagy gene, *IRGM*, nor within the *PTPN22* gene. The lack of variations within the *PTPN22* gene, which is also involved in the regulation of *NOD2* signalling and autophagy [15], in the CD patients is also of interest, as both of the variants are reported to protect from IBD [16].

Within the patient's family we found an interesting distribution of the risk variants with an accumulation in the CD-affected members: the index patient is hetero-

zygous for risk variants of the genes encoding the pathogen sensors *NOD2* and *TLR5*, and is a homozygous carrier of both of the *PTPN2* risk alleles. The CD-affected daughter carries one risk allele of both *TLR5* and *NOD2* and is heterozygous for both of the risk alleles of *PTPN2*. The son, with the earliest onset of disease in the family, has risk variants of all IBD-associated SNPs detected within the family: he is heterozygous for the risk alleles of *ATG16L1*, *TLR5* and *NOD2*, as well as both of the risk alleles of *PTPN2*. The father carries the risk variant of *ATG16L1* but none of the other analysed SNPs. The nonaffected daughters are both heterozygous for the risk alleles of *PTPN2* and carry either the risk variant of *ATG16L1* or *TLR5* but no variant of *NOD2* (Table 1). These observations seem to support the hypothesis that an accumulation of several genetic variations within an individual increases the risk for developing CD.

Interestingly, both of the children without CD carry a factor V Leiden mutation, which represents a genetic disorder characterized by a poor anticoagulant response to activated protein C and a high risk for venous thromboembolism. Deep venous thrombosis and pulmonary embolism are the most common manifestations. The clinical expression of a factor V Leiden mutation is influenced by a number of factor V Leiden alleles, coexisting genetic and acquired thrombophilic disorders and also circumstantial risk factors [17,18].

Thromboembolism is a well-described complication of IBD but knowledge about its possible role in the manifestation of this disease is still poor. The incidence fluctuates between 1 and 6% in clinical studies and 7 and 39% in autopsy studies [19–21]. The aetiology of thrombosis in IBD is multifactorial, but the exact mechanism remains unknown [22]. Several acquired prothrombotic risk factors include the inflammatory process *per se*, use of corticosteroids, surgical treatment, smoking and the use of oral contraceptives [23]. Genetic factors may also play a role in thrombosis in IBD patients. One of the most common genetic variants that affects the risk for thrombosis is factor V Leiden [21,24]. During the last few years there have been several studies assessing whether there is an association between factor V Leiden mutation and CD: in the study by Liebman *et al.* [21], a factor V Leiden mutation was found in 36% of 11 IBD patients with a history of thromboembolism and in 4% of 51 IBD patients

with no history of thromboembolism. Another study showed that factor V Leiden and prothrombin G20282A gene mutations were increased in IBD patients with a history of thromboembolism compared with IBD patients without a history of thromboembolism [21].

However, the limitation of our study is that we only present a single case of an interesting accumulation of CD cases in one single family. The two daughters without CD have a rather similar pattern of SNPs compared with the one daughter diagnosed with CD. Therefore, from our study, it is not possible to propose definite associations between the presence of a certain number of CD-associated SNPs and the onset of disease. Although our study highlights the role of genetic variations in the pathogenesis of CD, it is not sufficient to make significant associations between a genetic pattern consisting of a variety of CD-associated risk genes and the onset of CD.

Taken together, this family represents an interesting pattern of SNPs associated with CD and factor V Leiden. CD-affected family members accumulate several IBD-associated risk alleles of genes associated with the innate immune system. These observations clearly support the concept of genetic heritability in CD and underline the importance for further studies to identify the functional role of the IBD risk genes in the context of IBD pathogenesis.

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Conflicts of interest

There are no conflicts of interest.

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